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For: HYDROGENASE DEFICIENT BACTERIAL STRAINS

Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the aboveidentified application:

 (Currently Amended) An isolated bacterium selected from the group consisting of Salmonella, E. coli, Shigella, and and Campylobacter, wherein the isolated bacterium does not express a functional NiFe hydrogenase protein that has a greater than 75% reduction in hydrogenase activity relative to a wild type strain of said bacterium.

2. Canceled

3. (Currently Amended) The isolated bacterium of claim [[2]] 1 wherein the strain isolated bacterium comprises a mutation to each of the three NiFe hydrogenase genes present in the genome of the strain isolated bacterium, wherein each the mutation[[s]] prevents the expression of a functional NiFe hydrogenase protein.

Canceled

- 5. (Currently Amended) The isolated bacterium of claim [[2]] 1 wherein the bacterium is selected from the group consisting of Salmonella is Salmonella typhinurium[[,]] or Salmonella typhi, the E. coli is E. coli 0157, the Shigella is Shigella flexneri[[,]] or Shingella Shigella sonnei, and and the Campylobacter is Campylobacter jejuni.
- 6. (Currently Amended) A composition comprising an isolated bacterium selected from the group consisting of Helicobacter hepaticus, Salmonella typhimurium, Salmonella typhi, E. coli 0157, Shigella flexneri, Shingella Shigella sonnei, and and Campylobacter jejuni, wherein the isolated bacterium does not express a functional NiFe hydrogenase protein wherein said bacterium has a greater than 75% reduction in hydrogenase activity relative to a wild type strain of said bacterium.

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- (Currently Amended) An antigenic composition comprising an the isolated bacterium of claim [[3]] 1 and a pharmaceutically acceptable carrier.
- 8. (Original) The antigenic composition of claim 7 further comprising an adjuvant.
- Canceled
- (Original) The antigenic composition of claim 7 in the form of a frozen or lyophilized powder.
- 11. (Withdrawn-Currently Amended) A method of inducing an immune response in a mammal against a pathogenic bacterium said method comprising the step of administering to said mammal a composition comprising a live the isolated bacterium of claim 6.
- 12. (Withdrawn-Currently Amended) The method of claim 11 wherein the bacterium is selected from the group consisting of Salmonella typhimurium, Salmonella typhi, Helicobacter hepaticus, E. coli 0157, Shigella flexneri, Shingella Shigella sonnei, and and Campylobacter jejuni.
- 13. (Withdrawn) The method of claim 12 wherein the modification comprises a mutation to each of the NiFe hydrogenase genes present in the genome of the bacterium.
- 14. (Withdrawn-Currently Amended) A method of protecting a mammalian species against an infection with pathogenic Salmonella, E. coli, Shigella, or Campylobacter, said method comprising the step of administering to the subject a live bacterium, selected from the group consisting of Salmonella, E. coli, Shigella, and and Campylobacter, wherein the bacterium has been modified to prevent expression of a functional NiFe hydrogenase protein.

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- 15. (Withdrawn) The method of claim 14 wherein the live modified bacterium is administered orally at a dose of about 10⁴ to about 10⁸ cfu.
- 16. (Withdrawn) The method of claim 14 wherein the modification comprises a deletion mutation to each of the NiFe hydrogenase genes present in the genome of the bacterium.
- 17. (Withdrawn) The method of claim 16 wherein the mammalian species is protected from a Salmonella infection, said method comprising administering live Salmonella wherein each of the NiFe hydrogenase genes present in the genome of the bacterium has been mutated to prevent expression of a functional NiFe hydrogenase protein.

18. Canceled

- 19. (Currently Amended) The isolated bacterium of claim [[2]] 3 wherein said isolated bacterium is a mutant Salmonella typhimurium or Salmonella typhi, and wherein the isolated bacterium strain has comprises three deletion mutations, wherein the first deletion is flanked by nucleotides 1-136 and nucleotides 137-232 of SEQ ID NO:7, wherein the second deletion is flanked by nucleotides 1-289 and nucleotides 290-518 of SEQ ID NO:8, and wherein the third deletion is flanked by nucleotides 1-200 and nucleotides 201-333 of SEQ ID NO:9 made at STM 3147, STM 1538, and STM 1786 that prevent expression of the corresponding gene products.
- 20. (Currently Amended) The composition of claim 6 wherein the isolated bacterium comprises a mutation to each of three NiFe hydrogenase genes present in the genome of the isolated bacterium, wherein each mutation one or more NiFe hydrogenase genes of said bacterium have been mutated to prevents expression of the a functional NiFe hydrogenase gene product.

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- (Currently Amended) The composition of claim 20 wherein said composition comprises
 the <u>isolated</u> bacterium <u>is S. Typhimurium typhimurium</u> strain [[(]]JSG 321[[)]], deposited with
 the American Type Cell Culture <u>Collection</u> depository (10801 University Blvd, Manassas,
 <u>Virginia, 20108, USA), on February 4, 2005 and assigned deposit under Accession No: PTA6556.
 </u>
- 22. (New) The isolated bacterium of claim 1 wherein NiFe hydrogenase activity expressed by the cell is undetectable.
- 23. (New) An isolated bacterium selected from the group consisting of Salmonella, E. coli, Shigella, and Campylobacter that has decreased O₂-dependent H₂ oxidation activity relative to a wild type strain of said bacterium, and wherein the isolated bacterium has decreased virulence relative to a wild type strain of said bacterium when orally administered to mice.
- (New) S. typhimurium strain JSG 321 deposited with the American Type Culture Collection depository under Accession No: PTA-6556.